New generation of salmonella-based, single dose vaccine candidates to fight infant pneumonia

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One of the major challenges in modern vaccinology is to engineer vectors that are highly infectious, yet don't cause illness. Trickier still is to ensure that such weapons against infectious disease can be safely disarmed, once their immunogenic work is done. Roy Curtiss, an investigator of vaccines and infectious diseases at Arizona State University's Biodesign Institute, has pursued these goals for 30 years.

In his most recent study, published recently in the *Proceedings of the National Academy of Science*, Curtiss' research team unveils what may prove a winning strategy in the fight against infant bacterial pneumonia.

Two new vaccine strains designed in Curtiss' lab draw on the properties of an unlikely vaccine carrier—one generally associated with causing sickness rather than safeguarding the body against it. Salmonella typhimurium, a rod-shaped, motile pathogen is one of over 2000 strains or serotypes of the Salmonella constellation of bacteria. They are responsible for causing serious, sometimes fatal diseases, to which children under two years of age are particularly vulnerable.

Given this fact, Salmonella's choice as the principal component in a new vaccine for babies has been something of a hard sell. "People said 'you gotta be kidding,' " Curtiss recalls, noting that twenty years ago, Salmonella outbreaks were a grave concern in nurseries and hospitals, sometimes leading to the deaths of over half the children in such

facilities.

Salmonella strains are violently infectious, ransacking the body's defenses, as anyone who has suffered a bout of food poisoning can attest. Curtiss hopes to recruit Salmonella's appetite for infection and use it to speed delivery of a suite of key antigens—surface proteins of Streptococcus pneumoniae, causative agent of bacterial pneumonia. In the body, such antigens stimulate an immune response, but the additional pathogenic ingredients necessary to cause the disease are absent.

Such next-generation vaccine candidates offer new promise in the battle against S. pneumoniae, a prodigious killer causing more than 2 million annual fatalities worldwide.

The strategy of using a live bug like Salmonella to stimulate a protective immune response has been around awhile. But such microbes have typically had to be weakened or attenuated before safe use, disabling some of their virulence in order to prevent a full-blown occurrence of disease in the vaccine recipient. While the approach has been used with some success, Curtiss highlights the shortcomings of traditional attenuated vaccines: "If you make something safe and sort of cut off both arms and both legs, it can't get to where it needs to go in the body." Thus, attenuated strains, typically produced through deletion mutations of wild strains, may only produce local effects, failing to generate a powerful, system-wide immune response necessary for long-term protection.

In addition to triggering a powerful, protective immune response, Salmonella-based vaccines offer an inexpensive alternative which may be administered orally in a single dose—a significant advantage in the developing world.

Salmonella turns out to be a superb choice as an antigen delivery system.

Other infectious bacteria like Shigella, Vibrio cholera and pathogenic E. coli, all of which have been explored as vaccine candidates, only invade cells in the intestinal tract, failing to reach the liver and spleen, which are important workhorses for mounting an immune response. Curtiss further notes that intestinal cells turn over every 2-3 weeks, precluding long-term immunogenicity. Salmonella however, can spread throughout lymph tissues, spleen and liver, provoking system-wide immunity. Nevertheless, Salmonella vaccine strains produced through deletion mutations present many of the drawbacks of other attenuated forms, including reduced survival rate in the body's inhospitable environment, and depressed virulence.

Now, Curtiss and lead author Yuhua Li have led the development of two new vaccine candidates, labeled x9088 and x9558, under grants from the NIH and the Bill and Melinda Gates Foundation. These novel strains belong to a family known as recombinant attenuated Salmonella vaccines or RASVs. The critical component boosting their effectiveness is a delayed mechanism of attenuation. Salmonella's notorious virulence is essentially short-circuited, but only after it has stimulated a robust systemic immune response to pneumococcal surface protein A (PspA), a vital bacterial pneumonia antigen.

This feat is accomplished through genetic trickery to tame S. typhimurium, producing altered bacterial strains requiring mannose and/or arabinose—sugars available in the lab, but absent in the human body. After roughly 7 cell divisions, the bacterium exhausts its stores of specialized sugar. Unable to sustain the integrity of its cell wall, it bursts. By this method, Salmonella can be placed on a self-destruct timer, one that may be sensitively tuned to achieve maximum immunogenicity following colonization of host tissues. The technique was described in a paper from the Curtiss group with Wei Kong as the lead author, published last July in PNAS.

In comparison with attenuated Salmonella produced through deletion mutation, Curtiss' RASV delayed attenuation strains provoked significantly greater anti-PspA immune response (measured in serum antibody levels) as well as conferring greater protection from Streptococcus pneumoniae infection.

The safety aspect of self-destruct vaccines also makes them highly attractive. "We've got the Salmonella on a string," Curtiss says. "We can decide when to snap the string, and they're gone."

Indeed, in critical proof-of-concept mouse studies, a 20 percent higher protection rate was achieved even in the presence of a 10-fold increase in the challenge dose of pneumonia pathogen. Vaccine strain x9088 displayed a heightened ability to colonize the liver and spleen. In x9558, recombinant manipulation was used to delay not only virulence attenuation but also the onset of PspA synthesis—kick-started only in vivo, in an arabinose-free environment.

"We are now putting together all the protective protein antigens present on the surface of the pneumococcus," Curtiss states, adding that antigens for all 91 variant strains of S. pneumoniae will need to be incorporated to provide comprehensive immune defense from the disease.

An initial version of the new vaccine is slated to begin the first pre-clinical trials in human subjects, early in 2009.

Source: Arizona State University